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Original Article

Comparison of essential oil composition of *Stachys menthifolia* Vis. from two natural habitats in Croatia

Sanja Ćavar[1,2]*, Milka Maksimović[1], Marija Edita Šolić[3]

[1]*University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5, 1000 Ljubljana, Slovenia*
[3]*Institute „Mountain and Sea", Franjevački put 1, 21300 Makarska, Croatia*

E-mail: sanja.cavar@pmf.unsa.ba

***Abstract*:**
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Stachys menthifolia Vis. is an endemic species from the Balkan Peninsula. Aerial parts of the plant were collected from its natural habitat near Dubrovnik. Hydrodistilled volatile oil obtained from the plant material of *S. menthifolia* was subjected to gas chromatographic analysis coupled to mass spectrometry. More than 70 compounds were identified, representing 94.5% of the total oil. The major constituents of the oil were diterpenoid abietatriene (11.7%), and sesquiterpene hydrocarbons α-bisabolene (8.4%), and β-caryophyllene (7.4%). Presented results are comparable to our previous findings on essential oil composition of the same species from Biokovo Mountain, with small differences in quantitative and qualitative constitution of the oil. Although plants belonging to the *Stachys* genus show significant variability in their chemical compositions depending on the location and stage of plant development, this work indicates that chemical polymorphism of endemic *S. menthifolia* does not manifest in the region of Croatian Mediterranean area.

Key words: abietatriene, essential oil, GC-MS, *Stachys menthifolia* Vis.

Introduction

The genus *Stachys* is one of the largest representative genera of the Lamiaceae family, and includes about 300 species in the subtropical and tropical regions of both hemispheres (Mabberley, 1997). Like most of species belonging to the family Lamiaceae, *Stachys* species produce essential oils, but the composition of volatile compounds is known only in a small number of species (Maly, 1985; Cakir et al., 1997; Mariotti et al., 1997; Pélissier at al., 1999; Chalchat et al., 2001; Kukić et al., 2006; Palić et al., 2006; Radulovic et al., 2007). Available literature data indicate the existence of a chemical polymorphism of essential oil composition among *Stachys* taxa.

Stachys menthifolia Vis. is an endemic species of the West Balkan region. Šilić & Šolić (2001) discovered its natural habitat in the region of Biokovo Mountain in Croatia. The first location in Croatia was near Dubrovnik, discovered by Roberto Visiani, who was the first who described this species.

Continuing our phytochemical research on *S. menthifolia*, this paper presents the essential oil composition of the plant collected from its natural habitat near Dubrovnik.

Material and methods

Plant material was collected in May 2008 from the natural habitat near Dubrovnik. Voucher specimens have been deposited at the Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina.

All applied reagents were of the highest purity available and purchased from the Sigma-Aldrich Chemical Company.

Essential oil from the air-dried parts of *S. menthifolia* was isolated by hydrodistillation according to European Pharmacopoeia for 2 h. The oil was extracted with dichloromethane and dried over anhydrous sodium sulphate and stored at 4°C in the dark until the analysis.

Gas chromatography-mass spectrometry analysis was carried out on a Hewlett-Packard 6890 Series II gas chromatograph fitted with a fused silica HP-5 (5% phenyl methyl siloxane) capillary column (30 m × 0.252 mm, 0.25 µm film thickness), coupled to a HP 6890 Series II mass selective detector (MSD). Column temperature was programmed from 60°C to 240°C at 3°C min^{-1}, and helium was used as carrier gas. Other operating conditions were as follows: inlet pressure 9.43 psi, injector temperature 250°C, detector temperature 280°C, split ratio 1:25, injection volume 1 µL. Ionization of the sample components was performed in the EI mode, (70 eV), with scan range 20-555 amu, and scan time 1.60 s.

The linear retention indices, RI, for all compounds were determined by injection of the hexane solution containing the homologous series of C_8-C_{26} *n*-alkanes (Van Den Dool & Kratz, 1963).

The identification of the essential oil constituents was accomplished by the visual interpretation, comparing their retention indices and mass spectra with literature data (Adams, 2007) by computer library search (HP Chemstation computer library NBS75K.L, NIST/EPA/NIH Mass Spectral Library 2.0 and Mass Finder 4 Computer Software and Terpenoids Library), and in the laboratory own database.

Semi-quantitative analysis was carried out directly from peak areas in the GC profile.

Results and discussion

The yield of *S. menthifolia* hydrodistilled oil was 0.09% based on the dry weight of the plant material. Exactly seventy-six compounds were identified in the essential oil, representing 94.5% of the total oil.

The components identified in investigated sample of *S. menthifolia*, their retention indices, and percentage composition is summarized in Table 1. The major constituents of the oil were diterpenoid abietatriene (11.7%), and sesquiterpene hydrocarbons *α*-bisabolene (8.4%), and *β*-caryophyllene (7.4%). In general, sesquiterpenoid compounds predominate in the oil with 59.1%, where 35.1% are sesquiterpene hydrocarbons.

Presented results can be compared to essential oil composition of *S. menthifolia* from Biokovo Mountain (Ćavar et al., 2010), with some differences in quantitative and qualitative constitution of the oil (Figure 1). Oxygenated sesquiterpenes were the most abundant volatiles in *S. menthifolia* from Biokovo (Table 1), followed by diterpene hydrocarbons. This sample was characterized as the oil of abietatriene–8–*α*–acetoxyelemol chemotype. Significant differences between these samples were in the content of aromatic compounds, particularly 4'-methoxyacetophenone, which was not found in the population from Dubrovnik. Moreover, the content of sesquiterpene hydrocarbons was much higher in the sample from Dubrovnik. The observed differences might be explained by the fact that this plant material was harvested one month earlier than the sample from Biokovo.

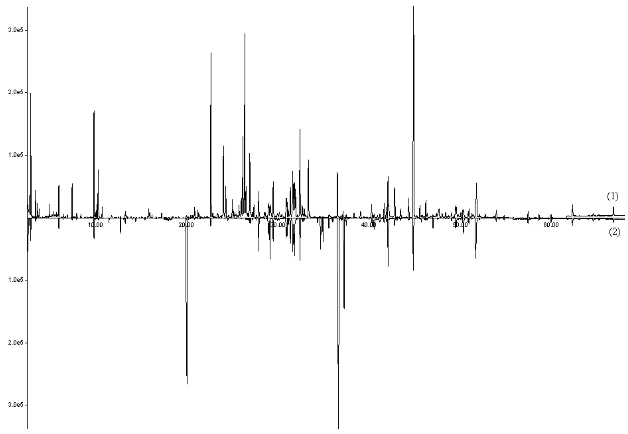

Figure 1. GC chromatograms of essential oil of *S. menthifolia* from Dubrovnik (1) and Biokovo (2)

Literature survey on studies related to the essential oils obtained from the plants belonging to *Stachys* genus from the Balkan Peninsula, showed significant variability in their chemical composition depending on location and stages of plant development. Essential oil obtained from *S. milanii* Petrović (Palić et al., 2006) had borneol and terpinen-4-ol as the most abundant compounds, while the major constituents of essential oil from Bosnian *S. alpina* L. spp. *dinarica* Murb. were *β*-

caryophyllene and germacrene D (Kukić et al., 2006). Dehydroabietane is the predominant compound in the essential oil of *S. plumosa* Griseb. (Petrović et al., 2006). Sesquiterpenoids were the main class of the constituents in Croatian *Stachys* species as follows: (*E*)-nerolidol in *S. alpina*; germacrene D in *S. officinalis*, *S. recta* subsp. *subcrenata, S. salviifolia*, and *S. sylvatica,* caryophyllene oxide, along with 1-octen-3-ol, in *S. palustris,* while *S. recta* subsp. *recta* contained *β*-ionone as the dominant volatile constituent (Bilušić Vundac et al., 2006).

Table 1. Comparison of essential oil composition of *S. menthifolia* from Croatia

#	RI	Compound	Dubrovnik RA[†] (%)	Biokovo[*] RA (%)
1.	801	Hexanal	0.3	tr[‡]-0.2
2.	847	(2*E*)-Hexenal	0.6	tr-0.2
3.	861	*n*-Hexanol	0.3	-
4.	874	2-Methyl butyl acetate	0.2	-
5.	930	α-Pinene	0.3	tr-1.0
6.	975	1-Octen-3-ol	0.9	tr-0.5
7.	1026	Limonene	1.0	0.1-3.4
8.	1041	Benzene acetaldehyde	0.1	tr
9.	1056	*γ*-Terpinene	0.2	tr
10.	1099	Linalool	3.6	0.7-1.7
11.	1103	Nonanal	0.2	tr
12.	1107	2-Methyl butyl isovalerate	0.5	-
13.	1111	1-Octen-3-yl acetate	1.9	-
14.	1122	3-Octanol acetate	0.4	-
15.	1189	*α*-Terpineol	0.3	tr-0.3
16.	1190	Methyl salycilate	tr	tr
17.	1255	Linalool acetate	0.3	-
18.	1286	*trans*-Linalool oxide acetate (pyranoid)	0.2	-
19.	1373	*α*-Copaene	0.5	tr-0.3
20.	1381	*β*-Bourbonene	0.3	tr
21.	1416	*β*-Caryophyllene	7.4	0.5-2.8
22.	1422	*β*-Maaliene	0.2	-
23.	1434	*β*-Guaiene	0.1	tr
24.	1450	*α*-Humulene	3.4	tr-0.2
25.	1456	(*E*)-*β*-Farnesene	1.4	-
26.	1478	*γ*-Curcumene	0.3	-
27.	1481	*ar*-Curcumene	0.3	tr-0.2
28.	1492	*α*-Muurolene	0.6	tr-0.2
29.	1499	(*E*,*E*)-*α*-Farnesene	1.3	-
30.	1503	*α*-Zingiberene	3.8	-
31.	1508	*α*-Bisabolene	8.4	tr-0.1
32.	1512	*γ*-Cadinene	1.7	tr-0.4
33.	1522	*δ*-Cadinene	3.3	-
34.	1530	*trans*-Cadina-1,4-diene	0.3	-
35.	1535	*α*-Cadinene	0.9	tr-0.2
36.	1547	Elemol	1.4	2.2-3.6
37.	1564	2-*epi*-(*E*)-*β*-Caryophyllene	0.3	-
38.	1574	Spathulenol	0.7	0.7-1.7
39.	1579	Caryophyllene oxide	tr	2.4-5.2
40.	1581	Clovenol	1.1	-
41.	1588	Globulol	1.9	1.5-2.4
42.	1600	Guaiol	tr	tr-0.3
43.	1606	*β*-Oplopenone	0.3	tr
44.	1627	*trans*-Isolongifolanone	0.9	1.2-1.6
45.	1629	*γ*-Eudesmol	0.9	1.1-1.9

#	RI	Compound	Dubrovnik RA[†] (%)	Biokovo[*] RA (%)
46.	1633	Caryophylla-4(15),8(13)-dien-5-*α*-ol	0.4	tr-0.4
47.	1640	*epi*-*α*-Murrolol	1.7	1.8-2.6
48.	1647	*β*-Eudesmol	2.3	2.4-5.0
49.	1650	*α*-Eudesmol	1.5	1.4-3.3
50.	1652	*α*-Cadinol	1.5	2.0-3.1
51.	1655	*allo*-Aromadendrene epoxide	1.3	-
52.	1669	Valeranone	4.5	-
53.	1676	*n*-Tetradecanol	0.5	0.5-0.6
54.	1682	*α*-Bisabolol	0.4	-
55.	1695	Isolongifolol	2.7	-
56.	1761	Benzyl benzoate	tr	-
57.	1788	8-*α*-Acetoxyelemol	2.1	6.9-21.3
58.	1807	Cryptomeridiol	0.3	0.5-6.7
59.	1844	6,10,14-Trimethylpentadecan-2-one	0.2	-
60.	1903	Isopimara-9(11),15-diene	0.6	0-5-1.1
61.	1954	Pimaradiene	0.5	0.2-0.8
62.	1963	*n*-Hexadecanoic acid	0.7	-
63.	1983	Manool oxide	1.9	0.7-2.3
64.	2002	(*E*)-Labda-7,12,14-triene	0.3	0.5-2.7
65.	2006	13-*epi*-Dolabradien	tr	0.3-0.6
66.	2050	Abietatriene	11.7	3.5-21.1
67.	2073	Abietadiene	0.6	tr-0.8
68.	2141	Abienol	0.4	0.7-0.9
69.	2206	2-Keto manool oxide	0.7	0.7-1.2
70.	2226	7-*α*-Hydroxy manool	0.4	0.2-0.3
71.	2258	Larixol	0.5	0.4-1.1
72.	2288	Dehydroabietal	1.9	1.5
73.	2298	*n*-Tricosane	tr	tr
74.	2499	*n*-Pentacosane	0.3	tr-0.2
75.	2599	*n*-Hexacosane	tr	tr-0.2
76.	2698	*n*-Heptacosane	0.6	-
		Aliphatic compounds	7.4	0.5-1.4
		Aromatic compounds	2.2	4.5-17.0
		Monoterpene hydrocarbons	1.5	0.1-4.6
		Oxygenated monoterpenes	4.4	1.7-2.9
		Sesquiterpene hydrocarbons	35.1	1.4-6.4
		Oxygenated sesquiterpenes	24.0	48.4-58.9
		Diterpene hydrocarbons	13.1	3.5-25.2
		Oxygenated diterpenes	6.8	1.5-5.8
		Total identified	94.5	86.8-90.8

[*]Data taken from Ćavar et al., (2010); [†]RA-Relative area; [‡]tr-trace (<0.1%).

Radulovic et al., (2007) suggested that high degree of variation in the main volatiles of the species of *Stachys* genus, especially of germacrene D and its congeners, was correlated with possible rearrangements under hydrodistillation conditions.

According to the presence and quantity of dominant compounds, the essential oil of investigated populations of *S. menthifolia* significantly differs from the previously published data concerning this species (Skaltsa et al., 2003). Greek *S. menthifolia* essential oil had abietatriene (13.7%), kaurene (9.0%) and 13-*epi*-manoyl oxide (7.5%) as the most abundant compounds. The observed differences in qualitative and quantitative composition of the essential oils between these two geographically isolated populations of *S. menthifolia,* confirm the influence of the environmental conditions on the volatiles found in plants. It is essential to notify that these prominent variations were not retrieved in the populations from Croatia.

Conclusion

In conclusion, GC-MS analysis on the essential oil of endemic *S. menthifolia* populations from Croatian Mediterranean area indicated similarities in qualitative, but small differences in quantitative composition of their essential oils.

Although plants belonging to *Stachys* genus show significant variability in their chemical compositions depending on the location and stage of plant development, this work indicates that chemical polymorphism of endemic *S. menthifolia* does not significantly manifest in the region of Croatian Mediterranean area.

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